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SHORT COMMUNICATION

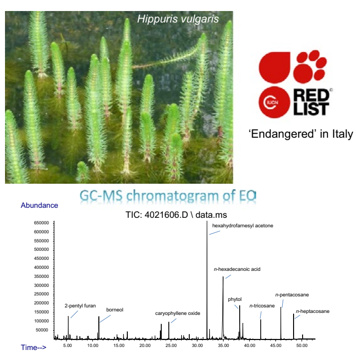
Volatile components of horsetail (*Hippuris vulgaris* L.) growing in central Italy

Kevin Cianfaglione[a,b], Fabrizio Papa[c] and Filippo Maggi[d]

[a]EA 2219 Géoarchitecture, UFR Sciences & Techniques, Université de Bretagne Occidentale, Brest, France; [b]School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy; [c]School of Science and Technology, University of Camerino, Camerino, Italy; [d]School of Pharmacy, University of Camerino, Camerino, Italy

ABSTRACT

Hippuris vulgaris, also known as horsetail or marestail, is a freshwater macrophyte occurring in lakes, rivers, ponds and marshes. According to 'The IUCN Red List of Threatened Species', *H. vulgaris* is at a high risk of extinction in Italy in the medium-term future. In the present study, we analysed for the first time the volatile composition of *H. vulgaris* growing in central Italy. For the purpose, the essential oil was obtained by hydrodistillation and analysed by GC-MS. The chemical composition was dominated by aliphatic compounds such as fatty acids (26.0%), ketones (18.7%) and alkanes (11.4%), whereas terpenoids were poorer and mostly represented by diterpenes (7.4%). *n*-Hexadecanoic acid (25.5%), hexahydrofarnesyl acetone (17.5%) and *trans*-phytol (7.4%) were the major volatile constituents. These compounds are here proposed as chemotaxonomic markers of the species.



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1. Introduction

Horsetail or Marestail (*Hippuris vulgaris* L.) is a freshwater macrophyte belonging to the Hippuridaceae family. It is an aquatic, creeping, perennial herb growing in Eurasia, America

CONTACT Filippo Maggi filippo.maggi@unicam.it
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and Africa. In some regions, the plant is disappearing as a consequence of pollution and destruction of habitat. In this regard, although *H. vulgaris* has been classified as 'Least Concern' in the majority of its range in the IUCN Red List of Threatened Species (Lansdown 2014), in some countries such as Italy it is considered as 'Endangered' (Rossi et al. 2013). According to 'The IUCN Red List of Threatened Species', a taxon is 'Endangered' when it is at high risk of extinction in the wild in the medium-term future (IUCN 2016).

In Italy, *H. vulgaris* is commonly called as 'coda di cavallo acquatica' because of the horse-tail-like shape of its emerging shoots (Pignatti 1982). It prefers non-acidic or base-rich waters (and mud) and it is found in lakes, rivers, ponds, marshes, bogs and ditches (Tutin et al. 1968). Its roots are underwater and emerge from stout rhizomes. The plant bears leaves in either aerial environment or underwater; in both cases they are 6–12 whorled; those submerged are thinner, limper and with a larger surface area than those above water. *H. vulgaris* can be found in two vegetative forms, waterlogged and emergent plants, respectively, with the former more common in spring and in flowing waters. The shoots are solid, un-branched and curved at the top; in shallow water, they emerge up to 30 cm from the water level. The flowers are ordinary and do not occur in all individuals.

Leaves and young shoots of *H. vulgaris* can be eaten raw or cooked (Uphof 1959; Usher 1974; Harrington 1988) and are used to make soups (Moerman 1998). They are generally harvested from autumn to spring, as well as the brown overwintered stems (Schofield 2003). The juice of the plant is applied externally and traditionally used as an effective vulnerary agent (Grieve 1984). The whole plant is also taken internally and used against tuberculosis and cough (Shang et al. 2012).

To the best of our knowledge, the *H. vulgaris* secondary metabolites have hitherto been scarcely investigated. Previously, only iridoids (aucubin, catalpol and their esters) and phenylethanoids (verbascoside) have been characterised in this species (Damtoft et al. 1994). On the other hand, no information is available about the volatile components.

Therefore, in order to find out volatile compounds with chemotaxomomic importance, in the present work, we analysed the essential oil of *H. vulgaris* by gas chromatography coupled with mass spectrometry (GC-MS).

2. Results and discussion

The volatile constituents occurring in the essential oil of *H. vulgaris* are reported in Table S1 (Supplementary Material). A total of 67 volatile components were identified, accounting for 83.5% of the total composition. The major fraction of the oil was given by aliphatic compounds (62.9%), with fatty acids (26.0%), ketones (18.7%), alkanes (11.4%), aldehydes (4.5%) and alcohols (2.1%) as the most representatives. The most abundant compounds were *n*-hexadecanoic acid (25.5%), hexahydrofarnesyl acetone (17.5%), *n*-pentacosane (4.0%), (2*E*,4*E*)-decadienal (1.0%) and (2*E*,6*Z*)-nonadienol (0.9%). Terpenoids were represented by diterpenes (7.4%), monoterpenes (5.3%) and sesquiterpenes (2.5%), with *trans*-phytol (7.1%), borneol (3.3%) and caryophyllene oxide (2.5%) as the most abundant compounds.

n-Hexadecanoic acid, also known as palmitic acid, is one of the most abundant fatty acids in the animal and plant kingdoms. Generally, it is found in high amounts in essential oil poor species, where it is often produced by hydrolysis of leaf waxes (Hargrove et al. 2004). This compound is endowed with *in vivo* anticancer activity in mice, by targeting the DNA topoisomerase I (Harada et al. 2002) and important antibacterial activity (Yff et al. 2002).

Other lipid degradation products were given by alkanes, aldehydes and alcohols. Notably, *H. vulgaris* essential oil was composed of long chain *n*-alkanes, with the linear odd carbon atom series C23-C27 as prevalent (*n*-tricosane, *n*-pentacosane and *n*-heptacosane accounting for 2.9, 4.0 and 2.8%, respectively). The odd *n*-alkanes are generated from cuticular waxes of epidermis tissues (Afshar et al. 2015). Also, aldehydes occurring in *H. vulgaris* essential oil, such as (2*E*)-hexenal (0.1%), *n*-heptanal (0.4%), (2*E*)-heptenal (0.1%), *n*-octanal (0.5%), (2*E*)-octen-1-al (0.4%), *n*-nonanal (0.4%), *n*-decanal (0.1%), (2*E*,4*E*)-nonadienal (0.1%) and (2*E*)-decenal (0.2%), may be formed from membrane lipids and polyunsaturated fatty acids (PUFA) due to free radical-mediated lipid peroxidation reactions (Luo et al. 1995).

Hexahydrofarnesyl acetone (6,10,14-trimethyl-2-pentadecanone) is a carbonylic compound derived from the diterpene alcohol phytol, and very common in the volatile fraction of plants. Notably, hexahydrofarnesyl acetone is produced by several orchids as a pheromone-like compound which is able to attract male orchid bees (Eltz et al. 2010). Its presence is typical of essential oil poor species (Maggi et al. 2009; Venditti et al. in press; Venditti et al. 2015).

Phytol is a diterpene alcohol formed from the degradation of chlorophyll occurring during distillation so that it is found in many essential oils. This compound has a balsamic odor and is used in the manufacture of vitamins E and K, soaps, detergent and beauty care products (Maggi et al. 2009). It has been discovered that the formation of this compound feeds biosynthesis of tocopherols in the plant cell (Mach 2015). Phytol has been shown as a promising anti-parasitic agent (da Silva et al. 2015). In addition, phytol inhibited the growth of *Pseudomonas aeruginosa* by inducing oxidative stress (Lee et al. 2016) and showed antimycobacterial activity comparable to that of clinically useful drugs (Rajab et al. 1998). Interestingly, phytol is endowed with important anti-quorum sensing and antibiofilm activities (Srinivasan et al. 2016). Furthermore, it is considered as a good antioxidant and cytotoxic agent (Costa et al. 2016; Thakor et al. 2016).

3. Experimental

See Supplementary materials.

4. Conclusions

The present study represents the first investigation on the volatile constituents of *H. vulgaris*, an aquatic plant locally endangered of extinction. Overall, our results provided new insights into the chemotaxonomy of the species. Furthermore, the presence of hexadecanoic acid, hexahydrofarnesyl acetone and phytol makes *H. vulgaris* potentially exploitable in the pharmaceutical industry.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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